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Short-Term Effects of South Louisiana and Kuwait Crude Oils on Glucose Utilization by Marine Bacterial Populations

STEVE K. ALEXANDER* AND JOHN R. SCHWARZ

Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77550

Two crude oils, South Louisiana and Kuwait, were examined for their impact on glucose utilization by bacterial populations from the Gulf of Mexico. The uptake and mineralization of [*U*-^{14}C]glucose was assayed after a 4- to 23-h exposure to various concentrations of added crude oil (0, 0.001, 0.01, and 0.1% [vol/vol]). The effects of oil were determined in a total of 15 sediment and 13 water samples collected from offshore, open-bay, and salt marsh environments. The utilization of glucose by bacterial populations usually was not affected by added oil; in 10 sediment and 11 water samples, oil had no significant effect on either glucose uptake or mineralization. Stimulation by oil was recorded in four sediment samples. Oil inhibition occurred in one sediment and two water samples, but only in the presence of the highest concentration of added oil, i.e., 0.1%. Our data suggest that short-term exposure to either South Louisiana or Kuwait crude oil, even at 0.1%, usually has no toxic effect on glucose utilization by marine bacterial populations.

An estimated 6.1 million metric tons of hydrocarbons enter the marine environment annually (10). The effects of oil input on marine bacteria have been examined in a number of studies (2-4, 8, 13, 16, 17). These investigations, however, have focused on oil-induced changes in the composition of the bacterial community, i.e., changes in population densities, physiological groups, diversity, and genera. Relatively few studies have examined the effects of oil on the activity of marine bacteria (7, 9). Therefore, the impacts of oil on nutrient regeneration processes in the marine environment are relatively unknown.

The present study was undertaken to examine the effects of two crude oils, South Louisiana and Kuwait, on glucose utilization by bacterial populations from offshore and estuarine environments of the Gulf of Mexico. The investigation involved the determination of [*U*-^{14}C]glucose uptake and mineralization rates after short-term exposure to various concentrations of added oil. Results suggest that South Louisiana and Kuwait crude oils usually have no impact on glucose turnover by marine bacteria at the concentrations employed.

MATERIALS AND METHODS

Sampling. Sediment and water were collected from five estuarine stations in the Galveston Bay system of Texas and from two sites off the Louisiana coast (Fig. 1). Estuarine stations 1, 2, and 3 were located in open-bay areas, whereas stations 4 and 5 were located in salt marsh habitats dominated by smooth cordgrass, *Spartina alterniflora*. Samples from offshore stations were collected during June, October, and November of 1978, and during January and May of 1979. Estuarine samples were collected during June of 1979.

Water was collected from mid-depth (offshore) or at a depth of 1 m (estuarine) with a Niskin sterile bag sampler (General Oceanics, Miami, Fla.). Submerged sediment was taken with either a Van Veen grab (offshore) or Ekman grab (estuarine). Emergent sediment (surrounding *S. alterniflora*) was collected during low tide with a sterile spatula. All samples were stored on ice until processing, usually within 6 h of collection.

Field sampling variables monitored during the study included: station depth, sample temperature (mercury-filled glass thermometer), sample salinity (Goldberg T/C refractometer, American Optical Corp., Buffalo, N.Y.), and total aerobic heterotrophic bacteria. Serial dilutions (in 0.1-ml volumes) of sediment or water were spread-plated in triplicate onto Marine Agar 2216 (Difco Laboratories). The inoculated plates were incubated at the seasonal in situ temperature for 10 days before bacterial colony enumeration.

Glucose uptake and mineralization. Sediment (10 ml of a 1:100 [vol/vol] dilution prepared in artificial seawater) and water (10 ml undiluted) were added to separate 30-ml serum vials (Wheaton Scientific). Sediment was added to a series of nine vials containing either 0.001, 0.01, or 0.1% (vol/vol) added South Louisiana crude oil (SL) in triplicate, and to another series containing Kuwait crude oil (K). In addition, sediment was added to triplicate serum vials containing no added oil (control vials, designated 0) and to triplicate vials containing one drop of concentrated H_2SO_4 (to correct for abiotic processes). Water was added to another group of serum vials similar to that described above for sediment.

After addition of samples, serum vials were sealed with a rubber stopper having a plastic center well (Kontes) suspended beneath it containing a 20- by

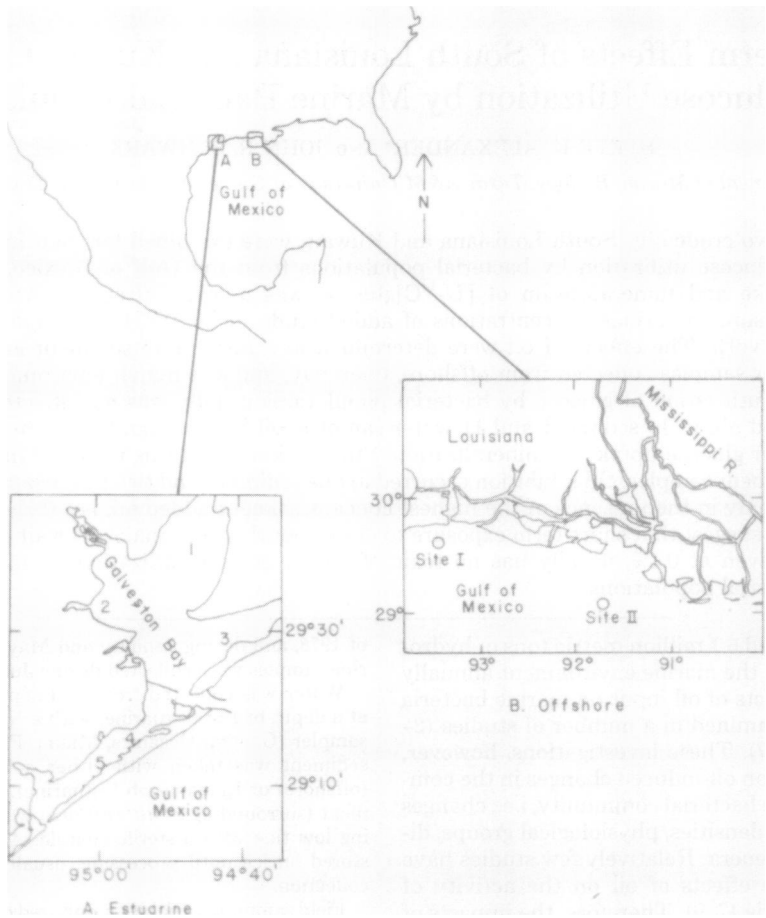

FIG. 1. *Sampling stations.*

60-mm piece of fluted Whatman no. 1 filter paper. Serum vials were incubated stationary at in situ temperatures for 4 to 23 h. After this period of exposure to oil, rubber stoppers were removed and 100 μg of [*U*-^{14}C]glucose (specific activity of 4.3 or 4.5 mCi/mmol; New England Nuclear Corp.) per liter was added to each serum vial. Vials were then resealed and incubated in the presence of radioisotope for an additional 1 to 3 h.

The $^{14}CO_2$ and bacteria in serum vials were then collected using methods similar to those of Hobbie and Crawford (6). One drop of concentrated H_2SO_4 was added to the liquid in serum vials via a syringe needle to terminate the reaction and drive $^{14}CO_2$ out of solution. The $^{14}CO_2$ liberated was collected by injecting 0.3 ml of phenethylamine onto the filter paper in the center well. The serum vials were left undisturbed for 12 to 24 h to allow complete absorption of $^{14}CO_2$ by the phenethylamine. After this period, the filter paper was removed from the center well and placed into a liquid scintillation vial containing 10 ml of cocktail, 0.4% Omnifluor (New England Nuclear Corp.) in scintillation-grade toluene (Fisher Scientific Co.). The bacteria were then collected by filtration through a 0.4-μm-pore-size polycarbonate membrane (Nuclepore Corp.). The membrane was rinsed with 10 ml of artificial seawater before addition to a liquid scintillation vial containing cocktail.

The radioactivity in liquid scintillation vials was assayed with a Beckman LS 8100 liquid scintillation counter. Quenching was corrected by use of 5 μl of [^{14}C]toluene internal standard (activity of 4.7×10^5 dpm/ml; New England Nuclear Corp.). The mean radioactivity of the filter paper and membrane from the triplicate acid-killed vials was subtracted from that of the remaining vials in a group. The rate of glucose utilization for each serum vial was then calculated and recorded as disintegrations per minute of [*U*-^{14}C]glucose taken into the cell (uptake) and mineralized to $^{14}CO_2$ per milliliter of sample per hour.

Statistical analysis. Analysis of variance was used to detect significant differences in glucose utilization rates between treatments (i.e., 0, 0.001, 0.01, and 0.1% added oil). When significant differences were detected,

Dunnett's procedure was used to determine which oil treatment mean was significantly different from the control mean. Analysis of variance and Dunnett's tests followed standard statistical formulas and procedures (14). A level of 0.05 was set to establish a significant difference in all tests.

RESULTS

Station characteristics. Offshore station depths were 7 to 12 m, whereas estuarine station depths were 1 to 4 m. Sample temperatures were highest (29°C) during June and lowest (11°C) during January at offshore stations. Estuarine sample temperatures were 26 to 28°C during June. The salinity of water was 18 to 30‰ offshore and 1 to 14‰ in the bay. Low salinities in the bay were due to high river runoff before sampling. Sediment bacterial populations were from 2.0×10^3 to 1.6×10^7 per ml offshore, and from 4.0×10^6 to 5.2×10^7 per ml in the bay. Bacteria in water were from 1.7×10^2 to 4.8×10^3 per ml offshore, and from 1.1×10^3 to 8.5×10^4 per ml in the bay.

Effects of oil: offshore. Glucose mineralization by sediment bacteria was not affected by added oil in five of eight samples from offshore (Table 1). Glucose mineralization in the three remaining sediment samples was stimulated by either 0.01 or 0.1% added oil. Oil had no impact on glucose mineralization by bacteria in water from the eight offshore stations.

Glucose uptake by sediment bacteria was unaffected by oil in seven of eight samples from

TABLE 1. *Effects of various concentrations of oil on [U-^{14}C]glucose utilization by bacterial populations from offshore*[a]

Date	Station	Exposure to oil (h)	Sample	Mean mineralization rate[b]				Mean uptake rate[b]			
				0	0.001%	0.01%	0.1%	0	0.001%	0.01%	0.1%
	Site I										
27 Jun 78	1	4	S	2.70	3.05(SL)	2.77	3.03	4.80	4.59(SL)	4.79	4.46*
					3.04(K)	3.27	2.98		4.99(K)	4.30	4.48
			W	−0.15	0.23(SL)	1.23	0.43	1.81	1.55(SL)	2.06	1.57
					−0.52(K)	0.57	−1.00		1.49(K)	1.82	2.16
13 Oct 78	2	7	S	3.23	3.23	3.10	3.43	5.11	5.20	5.01	4.50
					3.11	3.26	3.26		4.83	5.07	4.76
			W	−0.52	0.18	−1.00	−1.00	0.64	1.43	1.00	0.85
					ND	ND	ND		0.84	0.40	0.81
2 May 79	3	23	S	3.74	3.96	3.85	4.27	4.03	4.06	4.05	3.97
					4.01	4.25	3.98		3.97	4.11	3.85
			W	2.82	2.73	2.78	2.77	2.53	2.40	2.45	2.35
					2.78	2.75	2.80		2.47	2.22	2.17
2 May 79	4	23	S	3.97	4.18	4.46	3.84	4.00	4.24	4.26	4.39
					4.32	4.15	4.11		4.13	4.09	4.22
			W	2.29	2.16	2.53	2.23	2.37	2.10	2.39	2.51
					2.33	2.55	2.66		2.09	2.45	2.26
	Site II										
29 Jun 78	1	4	S	2.59	3.00	3.05	3.16	4.34	4.33	4.33	4.31
					2.91	3.30*	2.88		4.37	4.31	4.63
			W	0.26	ND	ND	0.04	0.58	0.72	0.49	0.53
					ND	ND	0.11		1.74	0.62	0.38
10 Nov 78	2	5	S	3.14	3.20	3.62*	2.54	3.51	4.29	4.86	3.56
					2.32	3.64*	3.67*		4.00	4.42	4.70
			W	−0.70	−0.10	0.08	0.04	0.11	−0.22	0	−0.40
					−1.00	−1.00	−0.40		0.32	−0.70	ND
28 Jan 79	3	15	S	0.48	1.48	3.78*	2.94	4.76	4.28	4.05	4.13
					2.34	3.10	3.70		5.07	4.39	3.09
			W	0.57	0.69	1.02	0.58	1.39	0.53	0.36	0.28
					1.08	0.91	0.71		1.30	0.97	0.54
28 Jan 79	4	15	S	3.77	3.35	3.91	3.59	5.01	4.79	4.79	4.88
					3.75	2.89	3.88		4.90	4.74	4.55
			W	ND	0.45	ND	ND	2.57	2.34	2.22	1.16
					ND	0.63	ND		2.40	2.36	1.96

[a] Abbreviations: S, sediment; W, water; SL, South Louisiana crude oil; K, Kuwait crude oil.

[b] Log_{10} dpm ml^{-1} h^{-1} of [U-^{14}C]glucose taken into cells (uptake) and mineralized to $^{14}CO_2$. Asterisk indicates those values significantly different from the control ($P < 0.05$). ND, No data.

offshore (Table 1). A 55% inhibition by 0.1% SL was recorded in the remaining sediment sample (from station I/1). The addition of oil did not alter glucose uptake by bacteria in water from the eight offshore stations.

Effects of oil: estuarine. Glucose mineralization by sediment bacteria from the five estuarine stations was unaffected by added oil (Table 2). Likewise, the addition of oil to estuarine water had no significant effect on mineralization.

Oil had no impact on glucose uptake by sediment bacteria from four of the five estuarine stations (Table 2). Uptake was stimulated twofold by 0.001% SL in the remaining sample (from station 1). Glucose uptake by bacteria in water from open-bay stations (1, 2, and 3) was unaffected by added oil. However, glucose uptake by bacteria in water from salt marsh stations (4 and 5) was inhibited an average of 37% by 0.1% added oil.

DISCUSSION

The inhibition of glucose utilization by oil occurred infrequently (in 1 of 15 sediment and 2 of 13 water samples) and only in the presence of the highest concentration of added oil, i.e., 0.1%. Although infrequent, inhibition may depend on the type of sample and the ecosystem from which the sample was obtained, since glucose uptake by bacteria in water from both salt marsh stations was inhibited by oil. The addition of oil to sediment and water samples from offshore, open-bay, and salt marsh environments usually did not significantly affect either glucose uptake or mineralization. In four sediment samples, activity was actually stimulated by oil. These results suggest that SL and K, although toxic on occasion at 0.1%, usually have no adverse impact on glucose utilization by marine bacterial populations.

Hodson et al. (7) examined the effect of four oils (SL, K, no. 2 fuel oil, Venezuela Bunker C oil) on the uptake and mineralization of glucose by bacteria suspended in "Controlled Ecosystem Enclosures." In their study, all four oils inhibited glucose uptake and mineralization. However, SL and K were the least toxic of the four oils, inhibiting glucose uptake an average of only 15% at 800 μg/liter compared to 60% for the other two oils. The relative toxicity of oils to marine animals is similar, with no. 2 fuel oil being 100 times or greater more toxic than SL or K (1). Previous studies on bacteria and marine animals, therefore, support the low toxicity of SL and K observed in the present study.

Petroleum hydrocarbons vary tremendously

TABLE 2. *Effects of various concentrations of oil on [U-^{14}C]glucose utilization by bacterial populations from estuarine stations*[a]

Date	Station	Exposure to oil (h)	Sample	Mean mineralization rate[b]				Mean uptake rate[b]			
				0	0.001%	0.01%	0.1%	0	0.001%	0.01%	0.1%
Open bay											
13 Jun 79	1	22	S	3.75	3.76(SL)	3.76	3.77	4.47	4.79(SL)*	4.29	4.21
					3.70(K)	3.68	3.77		4.39(K)	4.45	4.65
			W	1.70	1.70(SL)	1.71	1.84	2.79	2.71(SL)	2.61	2.38
					1.70(K)	1.68	1.74		2.76(K)	2.76	2.59
13 Jun 79	2	22	S	3.82	3.75	3.78	3.75	4.31	4.26	4.11	4.15
					3.77	3.76	3.68		4.11	4.09	4.03
			W	2.00	1.82	1.79	1.81	2.47	2.34	2.22	2.17
					1.91	2.38	1.76		2.40	2.36	1.99
14 Jun 79	3	17	S	3.72	3.79	3.78	3.72	4.48	4.73	4.34	4.37
					3.81	3.75	3.73		4.55	4.60	4.62
			W	2.02	2.20	2.00	2.03	2.64	2.63	2.64	2.43
					1.97	1.95	1.87		2.61	2.58	2.56
Salt marsh											
14 Jun 79	4	17	SS	4.03	3.95	3.90	3.91	4.45	4.32	4.42	4.26
					3.97	3.85	3.99		4.56	4.34	4.33
			ES	3.86	4.09	4.00	3.96	4.45	4.56	4.65	4.46
					4.00	3.95	3.93		4.54	4.54	4.45
			W	2.04	2.10	1.96	1.91	2.35	2.44	2.25	2.10*
					2.10	1.95	2.00		2.34	2.36	2.22
19 Jun 79	5	5	SS	3.87	3.83	3.85	3.88	4.36	4.44	4.28	4.22
					3.92	3.84	3.87		4.36	4.42	4.24
			ES	3.86	3.83	3.88	3.84	4.11	4.07	4.17	3.94
					3.92	3.86	3.86		4.20	4.16	3.93
			W	1.79	1.77	1.66	1.69	2.07	2.01	1.85	1.81*
					1.79	1.69	1.56		2.01	1.93	1.66*

[a] Abbreviations: SS, submerged sediment; ES, emergent sediment; others as in Table 1.
[b] Log_{10} dpm ml^{-1} h^{-1} [U-^{14}C]glucose taken into cells (uptake) and mineralized to $^{14}CO_2$.

in their toxicity to marine organisms (1, 7, 11, 12). For example, no. 2 fuel oil and Bunker C oil are more toxic than SL and K due to a higher concentration of naphthalenes in the former oils (1, 7). Therefore, results from the present study on the toxicity of SL and K may not be representative of other oils. Results obtained may well depend on the type of oil used.

The most frequently used indicator of heterotrophic activity in the aquatic environment is V_{max} (15). Determination of V_{max} involves the use of several concentrations of radiolabeled substrate to calculate a maximum potential utilization rate independent of substrate concentration. The present study and others (5, 7) have used a single concentration of radiolabeled substrate to determine the effect of pollutants on rates of substrate utilization. While this approach is useful for comparison of utilization rates in control and perturbated samples, it does not allow calculation of V_{max}. Therefore, it is possible that V_{max} could have been altered but not detected by the methods employed in this study.

One point of discussion is the change in bacterial populations that may have occurred during the short-term exposure to oil (<23 h). Marine bacterial populations exposed to chronic oil inputs increase in abundance, especially those bacteria capable of degrading hydrocarbons (2, 8, 13, 16). It is not known if a stimulation of hydrocarbon-degrading bacteria occurred in the samples during short-term exposure to oil. If so, there may have been an inhibition of certain heterotrophic bacteria which was not recorded due to a stimulation of others, i.e., hydrocarbon degraders. Even if this did occur, the total utilization of glucose was unaffected by oil in most samples.

Although short-term exposure to oil did not adversely effect glucose utilization in most samples, this may not be the case with chronic exposure. Chronic oil input, with resultant changes in the composition of the bacterial community (cited previously), may effect rates of glucose utilization. Therefore, the effects of long-term exposure must be ascertained before it can be stated conclusively that SL and K have little toxic effect on glucose turnover by marine bacterial populations.

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